T. neapolitana DLE (2779) SOM

Project No. 20222
Book No. 3884

Page No. 48

October 13, 1994 (Thursday)

+ infected DH12S cells with the ϕ from #5, 6, 7 (2ml cells grown in 2X YT + 3μl ϕ; grown 37°C (air shaker) 16 hrs)

RF isolated by alkali/SDS except 1μl RNase A (1mg/ml) added to prep at DNA ONLY addition

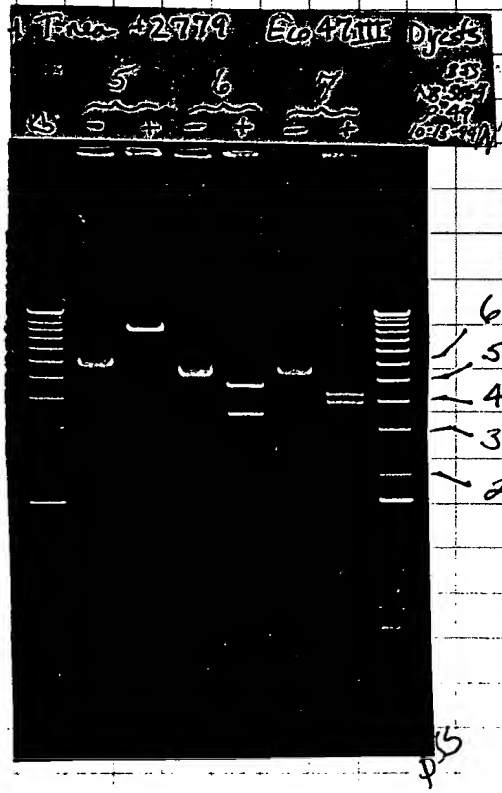
DNA dissolved in 50μl T10E1

DIGEST SCHEME

	H2O	10μl	✓
(React 3)	10X B/R	2	✓
	DNA	7	✓
4U/μl	Eco47III	1	✓
	TOTAL	20μl	

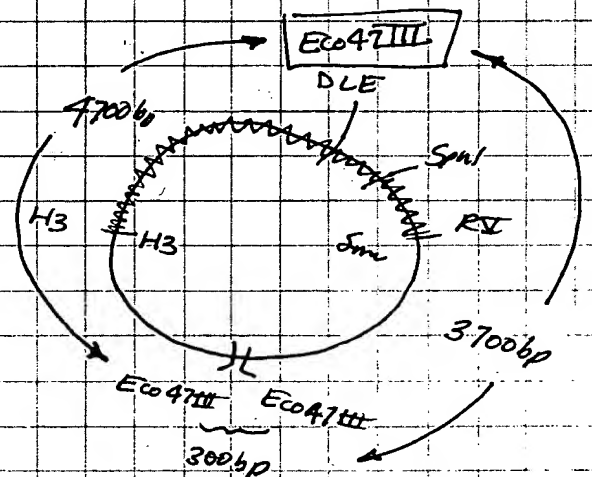
Incubated 37°C (Heat block) 1 hour.

3% Agarose Gel (1XTAE); 190V 1h



6 Kb
5 Kb
4 Kb
3 Kb
2 Kb

Comments:



The bands are migrating at the expected distances for #6. There must have been an overabundance of some "component" causing the DNA to run faster. I will clone into the pUC vector.

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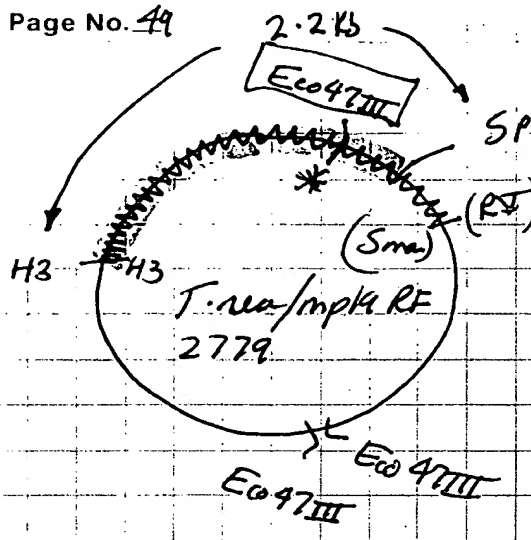
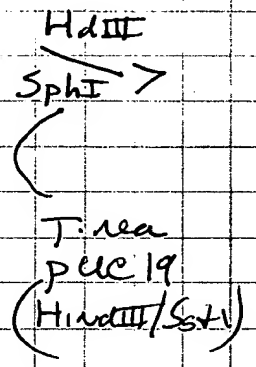
Understood & Understood by me,	Date	Invented by	Date
Mary Forgo	10/24/94	Record'd by Brian J. Schmidt	10-13-94

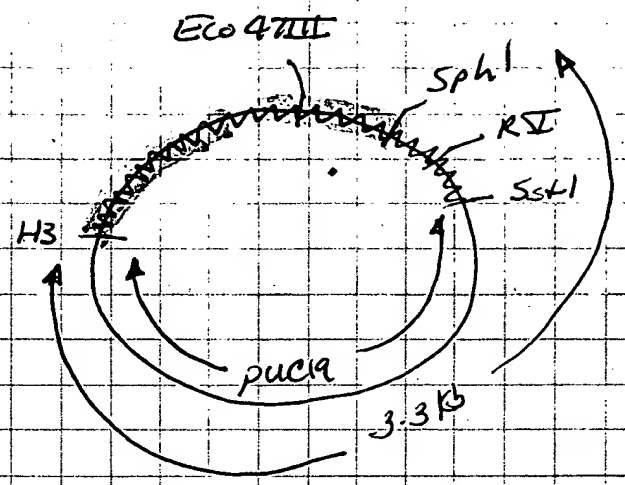
Project No. 2022
Book No. 3884 TITLE T. neapolitana DLE (2779) SDM

From Page No. 49

October 12, 1994 (Thurs





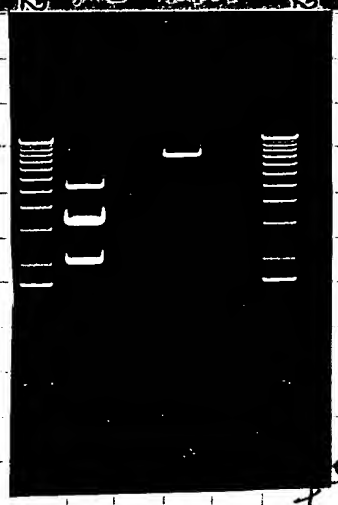


DIGEST SCHEME

		T.nea/pUC		#6 2779/mp19		
	H2O	12 µl	✓	6 µl	✓	Incubated 37°C (heat-
(React 2)	10X Bfr	2	✓	2	✓	1:10 → 1:50
	DNA	4	✓	12	✓	
(10 u/µl)	HindIII	1	✓	1	✓	
(10 u/µl)	SphI	1	✓	1	✓	
	Total	20 µl		20 µl		

0.8% Agarose Gel (1XTAE) 190

10/24/94



Comment: I should see a 3.3 Kb (desired fragment) and a 2.2 Kb fragment from the T.nea/pUC19 clone and I do. Unfortunately I should see a 7.9 Kb fragment and 2.2 Kb (desired fragment) from the T. nea/mp19 (2779 #6) RF DNA and I don't. Both sites were present before I performed the mutagenesis (see p. 35) - I will have to repeat.

To Page No

Witnessed & Understood by me,	Date	Invented by	Date
	10/24/94	Recorded by	10-13-94

ge N .__

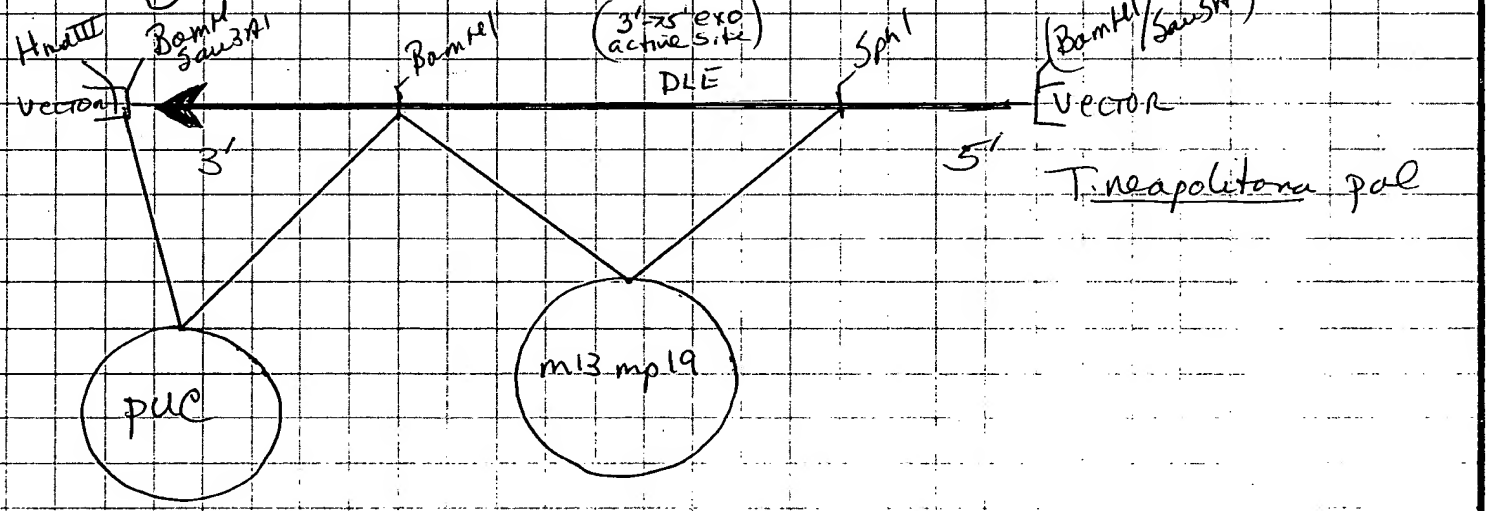
January 25, 1995 (Wednesday)

I'M BAAACK!!

After a tour of duty with Joe's group, a trip to Aruba and a few sneek vacation days I am back and ready to administer the fatal blow to this project. I will finish sequencing this gene, mutagenize it to conform to our needs, and overexpress it so people can get enough enzyme to swim in it and still have money left over for a cup of coffee and a copy of the New York Times!

How 'bout for an opening!

First things first. Lets reclone the region of the pol gene we are interested in mutagenizing. Bob and I have had no success with the last clone. Secondly, lets make the subclone more user friendly by eliminating the 3' BamHI site.



stray BamHI/Sau3A PCR
clone Hd3/BamHI

make SSDNA
D -> A by SDM

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ed & Understood by me,	Date 1/27/95 4/20/95	Inv nt d by	Date
		Recorded by	1-25-95

From Page No. 51

January 25, 1995 (Wednes.

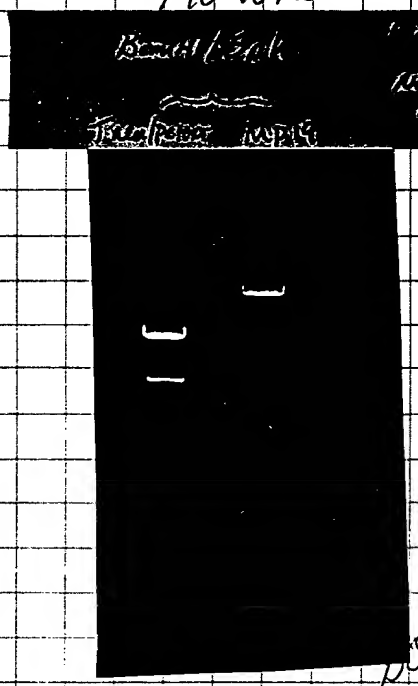
T. neapolitana /pSPORT DNA made by Michael Smith (no of the Nobel Laureate; the Hosebag)

DIGEST SCHEME

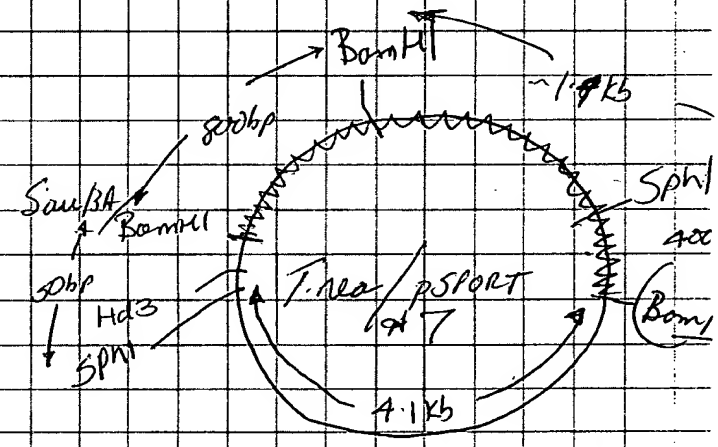
	T. nea/pSPORT		mBmp 19		pUC 18	
H$_2$O	15 µl	✓	13 µl	✓	13 µl	✓
(React 6) 10 × B/R	2	✓	2	✓	2	✓
DNA	1	✓	3	✓	3	✓
(10u/µl) BamHI	1	✓	1	✓	1	✓
(10u/µl) SphI	1		1		1	
Total	20 µl		20 µl		20 µl	
(0.1u/µl) CAP					1 µl	

Incubated 37°C (heat-block) 1:00 → 2:45

0.8% Agarose Gel (1×TAE)
190 Volts



11/27/95



I forgot to run the 1Kb ladder. what's at hose bag!

Fragments T. nea/pSPORT 7 BamHI/SphI should be 4.5 Kb, 1.4 Kb, 0.8 Kb, 0.05 Kb.
Perhaps something pooballed. Try again but do it separately.

Witnessed & Understood by me,	Date	Invented by	Date
	11/27/95	Recorded by Frank Schmidt	1-25-95

January 26, 1995 (Thursday)

DIGEST SCHEME:

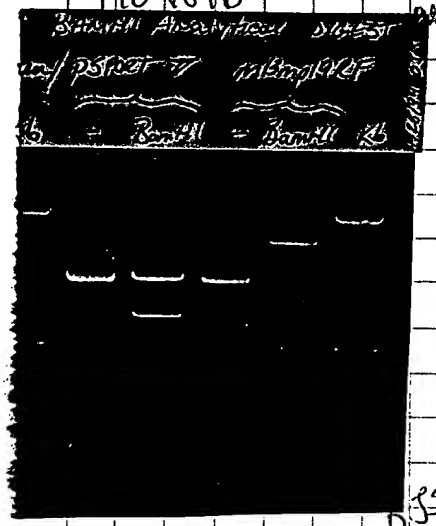
		T.nea/pSPORT		m13 mp19 (~270ng/ul)	
	HOH	24.5 ul	✓	22.5 ul	✓
] (React 3)	10XB/R	3	✓	3	✓
	DNA	1	✓	3	✓
F107] (10U/ul)	BamHI	1.5	✓	1.5	✓
	TOTAL	30 ul		30 ul	

Incubated 37°C (heat block) 8:04 → 9:08

✓3 ul removed for analytical gel.

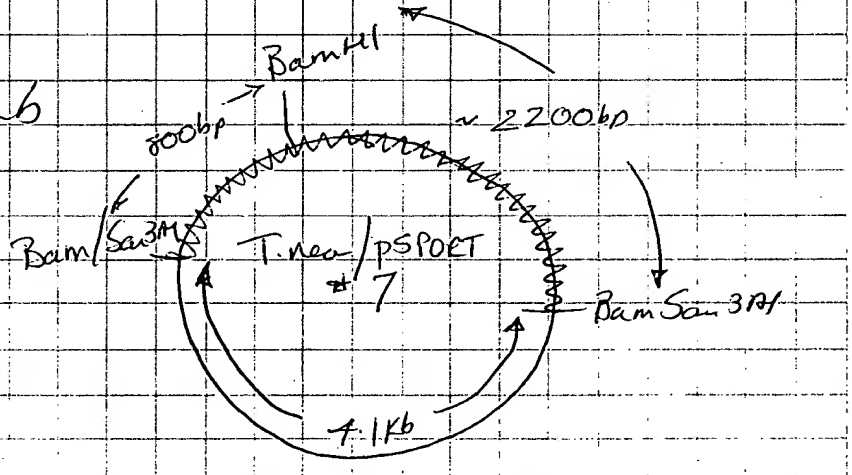
		T.nea/pSPORT	mp19	
DIGEST	27 ul	✓	✓	Incubated 37°C (heat block) 9:17 → 10:25
1M KCl	2	✓	✓	
HOH	9	✓	✓	
BamHI	2	✓	✓	
TOTAL	40 ul			

✓ Agarose Gel (1XTAE) 190 V o 1h



DJS 11/27/95

Comments



To Page No.

Signed & Understood by me,	Date	Invented by	Date
Mary Torpey	1/27/95	Recorded by Dennis J. Schmidt	1-26-95

[redacted]

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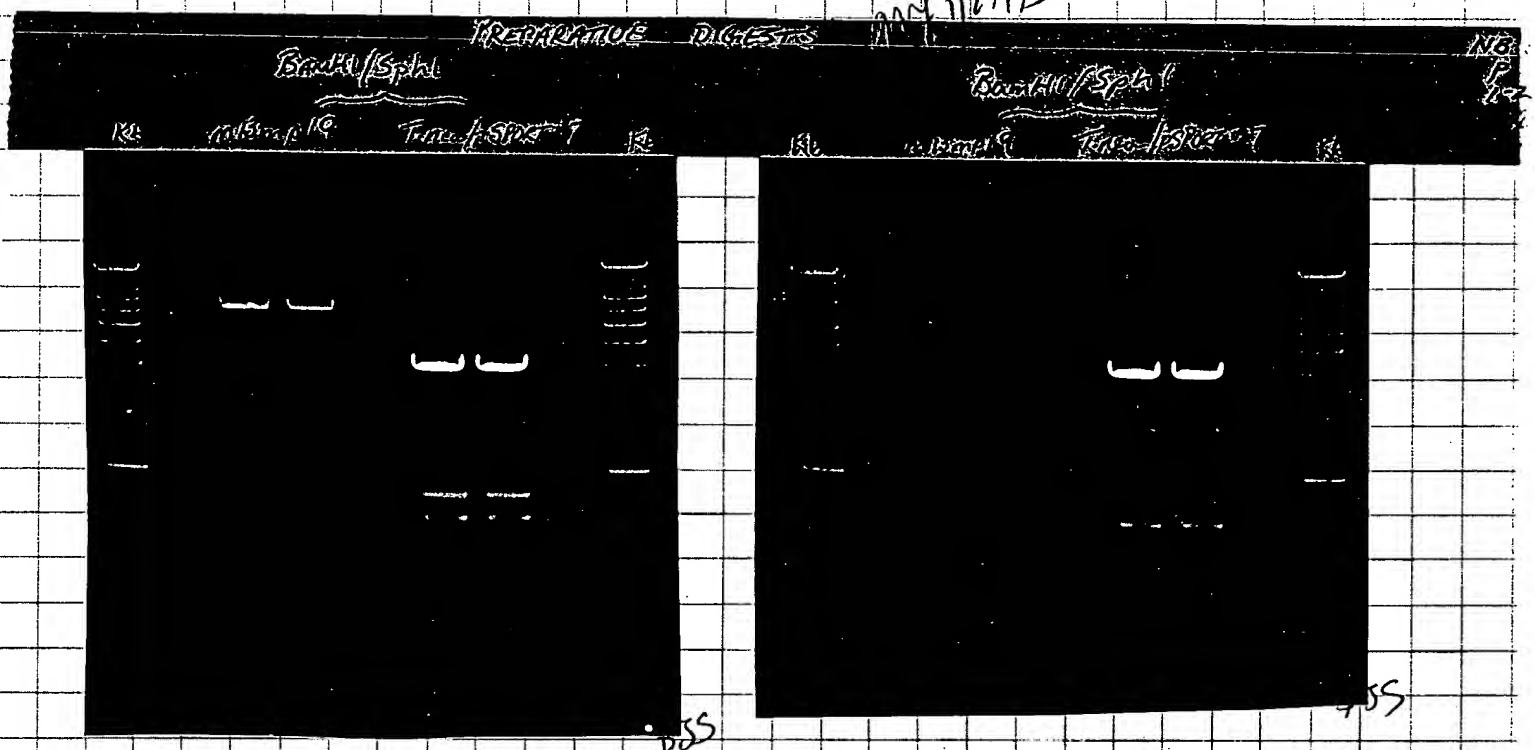
January 26, 1995 (Th

0.8% Agarose Gel (1x TAE); Run at 190 Volts

11/27/95



Bands extracted from the gel and placed in the same tube. The DNA was purified away from the agarose and then clean as described by the manufacturer (B10-101)

DNA eluted in 14 µl HOH

LIGATION SCHEME

	DNA	14 µl	✓
EE6402 (Ligase)	5X B/R	4	✓
1/4 µl (Ligase)		2	✓
	Total	20 µl	

Incubated 22°C (room-temp)
2:15 → 3:15

↳ 1 µl ligation / 2 µl for transformation

To Page

Witnessed & Understood by me,	Dat	Invented by	Date
Mary Tompkins	11/27/95	Recorded by Douglas J. Schmidt	1-26-95

age No. 54

January 26, 1995 (Thursday)

DH10B Electroporation

20 µl DH10B Electrocompetent Cells + 1 µl (of a 1/3 dilution, Sep 54)

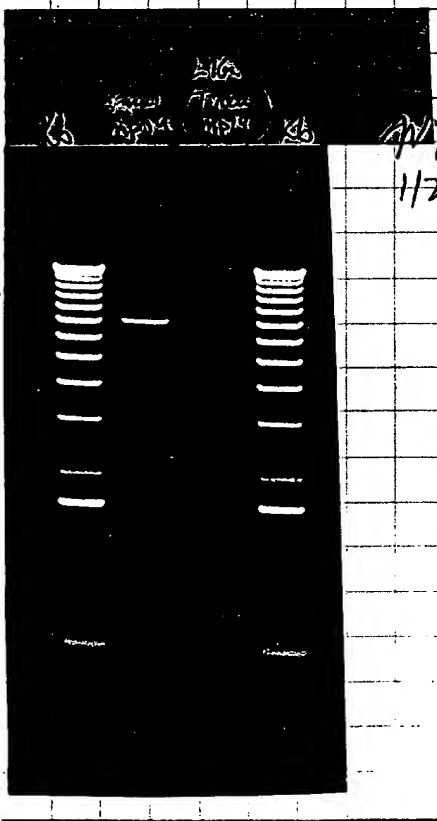
2.5 KV

1 ml LB, 37°C air shaken 20 min

↳ 10% applied to LB plate in 4 ml Soft Agar (0.7%)

90% + IPTG (1mM) and X-Gal 100 µl of 4%

Incubated 37°C incubator



MM
11/29/95

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ssed & Understood by me,	Dat	Invent d by	Date
Mary Longo	1/27/95	R corded by Brian J. Schmidt	1-26-95

Project No. 20222
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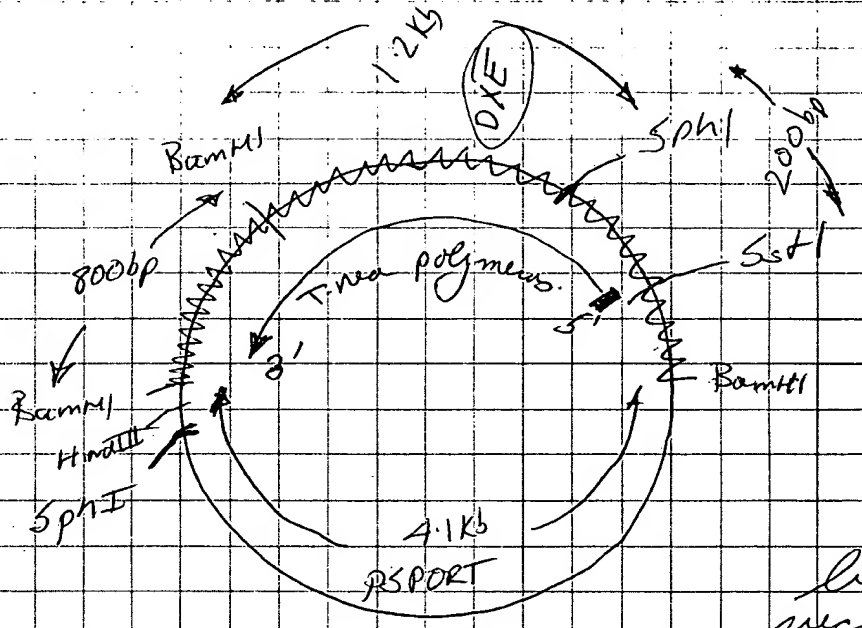
February 7, 1995 (Tuesday)



I can clone the 5phI fragment into m13mp19 and let m13 determine which direction is best suited.

I can subclone the 4.1Kb into an expression vector with 5phI/HindIII

DIGEST SCHEME

	T-neo PSPORT		(272ng/μl) mB mp19		
HOH	21 μl	✓	20 μl	✓	9:42 am →
(Read 6) 10X Buf	3	✓	3	✓	
DNA	3	✓	3	✓	
[CMG105] (10U/μl) 5phI	3	✓	3	✓	10
(0.1U/μl) CAP	0		1	✓	9:46 am →
TOTAL	30 μl		30 μl		

Run on 0.8% Agarose Gel at 75 Volts

To Page

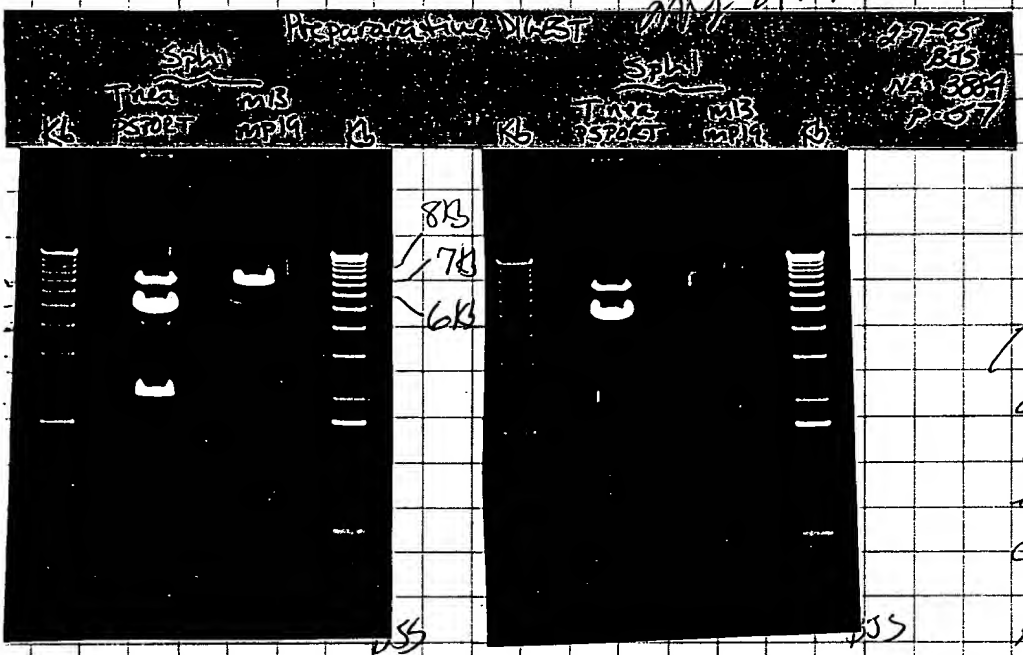
Witnessed & Understood by me,	Date	Inv nted by	Date
Mary Longo	2/16/95	Recorded by Paul F. Schmidt	2-7-95

g No. 56

February 7, 1995 Tuesday

0.8% Agarose Gel (1X TAE), 25 Volts.

2/16/95



Bands extracted from the gel and the DNA purified away from the agarose using Gene Clean as described by BIO-101.

DNA eluted in 14 µl HOH

LIGATION SCHEME

HOH	—	µl
5X B/R	4	✓
DNA	14	✓
4 U/µl T4 DNA Ligase	2	✓
TOTAL	20 µl	

Incubated 3:23 pm → 4:03 at room-temperature (~22°C)

↳ 3 µl removed for transformation

152 F' IQ Competent Cell Transformation
0 µl competent cells + 3 µl ligation (see above)
2 min on ice, 35 seconds at 42°C water bath,
1 and 90 µl applied to LB + No Antibiotic plates in
4 ml 0.7% Top Agar + 100 µl 2% X-Gal + 10 µl 100 mM IPTG
incubated 16 hours at 37°C incubator

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Read & Understood by me,	Date	Invented by	Date
Nancy Longo	2/16/95	Recorded by [illegible] Schmidt	2-7-95

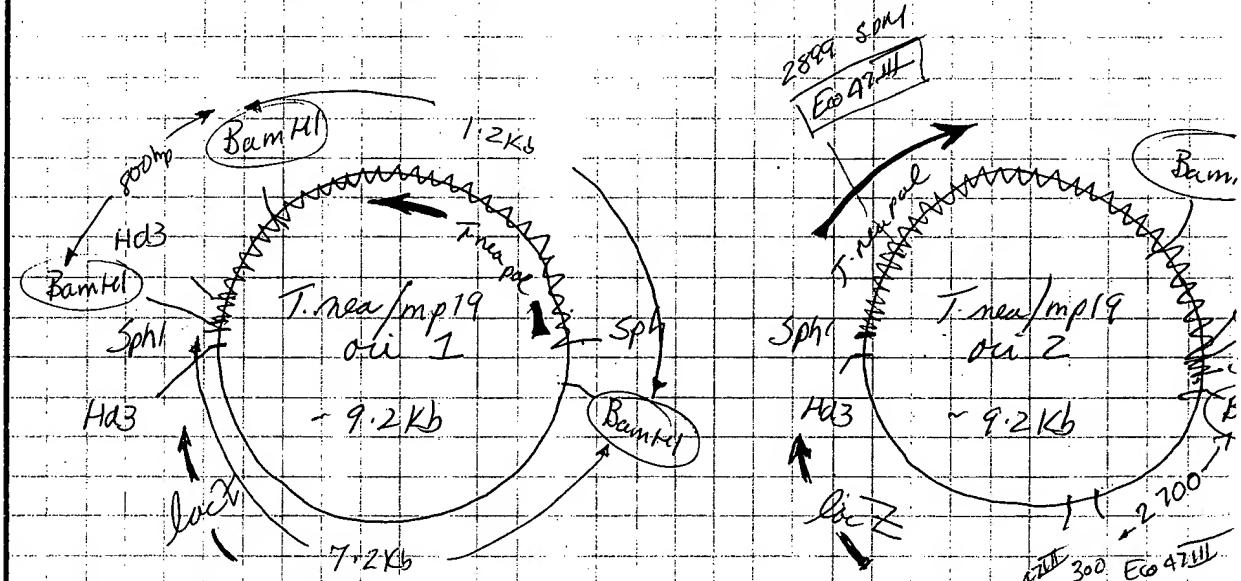
Project No. 2022L

58 Book No. 3884 TITLE T. neapolitanei SDM

From Page No. 57

February 8, 1988 (Mon)

→ I added 200 µl of DH5αF'IQ lawn cells to 10 ml Circle Grow.
→ I added 1 ml of the cells to 8 glass tubes
→ Each tube was inoculated with a clear plaque and incubated at 37°C (8:00 am →



SphI	7.2 Kb	VECTOR		7.2 Kb	VECTOR
	2 Kb	INSERT		2 Kb	INSERT
BamHI	7.2 Kb	VECTOR		8.4 Kb	VECTOR+I
	1.2 Kb	INSERT		0.8 Kb	INSERT
	0.8 Kb	INSERT			

Add T. nea/pSPORT as a positive control for both digests

To Page

Witnessed & Understood by me,	Date	Invented by	Date
Mary Torres	2/11/88	Recorded by	2-8-88

ag No. 58 February 8, 1995 (Wednesday)

DIGEST SCHEMES

1 —

	PER RXN	x 9 =	COCKTAIL	
HOH	7 µl	x 9 =	63 µl	☑
React 6) 10x B/H	2	x 9 =	18 µl	☑
DNA	10			
(10 µl/µl) Sph I	1	x 9 =	9 µl	☑
Total	20 µl		90 µl	

For Indianapolis/PSIORT continue add

To E, 20 µl ☐
DNA 2 µl ☐
Total 22 µl ☐

✓ add 10 µl to reaction

	PER RXN	x 9 =	COCKTAIL	
HOH	7 µl	x 9 =	63 µl	☑
React 3) 10x B/H	2	x 9 =	18	☑
DNA	10			
(10 µl/µl) BamHI	1	x 9 =	9	☑
Total	20 µl		90 µl	

Continued on page 1 of Notebook 3966

Anne - Patel Cell photo

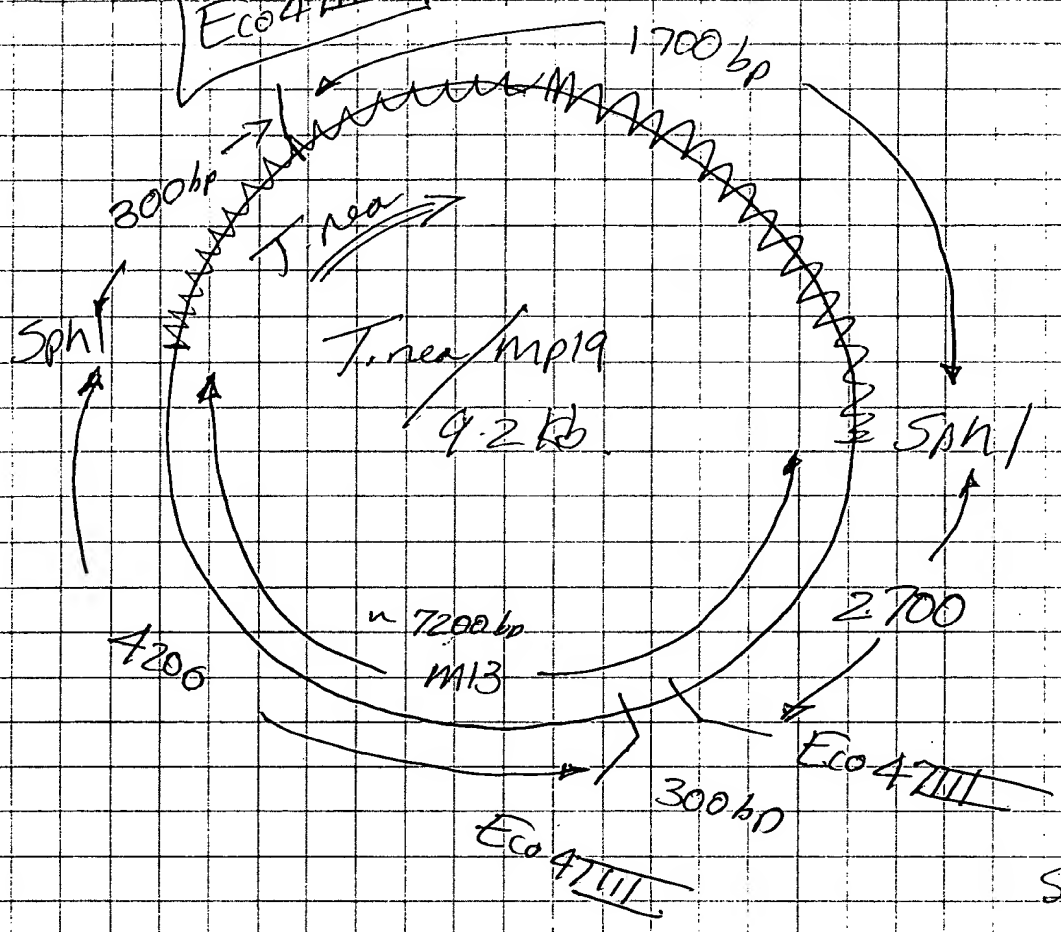
To Page No.

sed & Understood by me,	Date	Invented by	Date
Mary Tompo	2/16/95	Recorded by Brian J. Schmidt	2-8-95

age No.__

SDM 2899

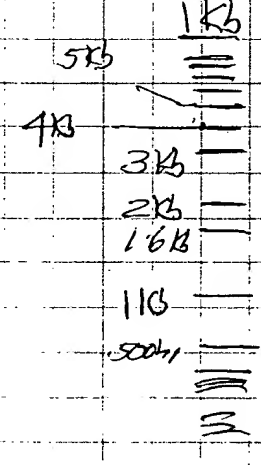
RF map



See on fil

PARENT

Eco 47III
8.9 Kb
0.3 Kb



PARENT MUTANT

MUTANT

4.5 Kb
4.4 Kb
0.3 Kb

may be too light

To Pag No.____

sed & Understood by me,	Date	Invented by	Dat
Mary Longo	2/16/95	Record d by: Lynn J. Schmidt	2-16-95

Tne mutant Phe to Ala

g No.

① The same phenyl alanine corresponding to Taq polymerase ill be changed to tyrosine

② For exo D will be changed to Alanine (corresponding region of Taq).

rian cloned the SphI fragment of Tne Pol into M13mp.

I isolated the single stranded DNA from CJ236 as escribed before in Bio rad manual.

Test 5µl ssDNA

The DNA looks real good.

For D→A (3'-5' exo mutant oligo) is

5' GA | CGT | TTC | AAG | CGC | TAG | GGC | AAA | AGA # 2899

Eco47III site

For Phe → Tyr (O-helix) HpaI.

~~GAA~~ GTA | TAT | TAT | AGA | GTA | GTT | AAC | CAT | CTT | TCC | A

2904

ian kinased 2899 before.

kinased 2904 as follows: 2µl oligo (210 pmol)
6µl 5x buffer (350mM Tris pH 7.6, 50mM $MgCl_2$, 50mM KCl, 5mM BME)
1µl 10mM ATP
0.5µl T4 Kinase (5U)
20.5µl H_2O
5' at 37°C → Heat at 65°C + 30µl TE.

T Page Maze

sed & Und rstood by m ,	Date 4/6/95	Invented by	Date 3/14/95
		Record d by	